

# Identification and Characterization of Two Distinct Bursal B-Cell Subpopulations Following Infectious Bursal Disease Virus Infection of White Leghorn Chickens

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Received 22 August 2008; Accepted and published ahead of print 31 March 2009

**SUMMARY.** Infectious bursal disease virus (IBDV) is an immunosuppressive virus which primarily infects IgM B-cells in the bursa of Fabricius. Flow cytometric analysis was used to phenotype B-cell populations in the bursa and spleen following IBDV infection. In the bursa, two IgM B-cell subpopulations, designated as A and B, were identified based on cell size and granularity. While both subpopulations differentially expressed IgM and Bu-1b surface markers, both groups displayed major histocompatibility complex class II surface antigens at equal levels. Following IBDV challenge of nonvaccinated birds, the B subpopulation was significantly reduced between 7 and 21 days postchallenge compared to either nonchallenged birds or vaccinated–challenged birds. However, the reduction of subpopulation B in the bursa, following IBDV exposure, did not reduce the levels of total serum IgA, IgG, and IgM, nor did it affect IgG and IgA B-cells in the spleen. Phenotypic analysis of the subpopulations identified differential expression of Lewis<sup>x</sup>, IgM, Bu-1b, and MUI78 surface antigens between the subpopulations. Overall, these are the first studies to identify two distinct IgM B-cell subpopulations in the chicken bursa, and the first to describe the decrease in the IgM B-cell population relative to IgA and IgG B-cells following IBDV infection.

**RESUMEN.** Identificación y caracterización de dos distintas subpoblaciones de células B en la bolsa de Fabricio después de la infección con el virus de la enfermedad infecciosa de la bolsa en aves Leghorn.

La enfermedad infecciosa de la bolsa es una enfermedad inmunodepresora que afecta primariamente células B que se encuentran en la bolsa de Fabricio y que presentan en su superficie moléculas de IgM. Se utilizó el análisis por citometría de flujo para la fenotipificación de las poblaciones de células B en la bolsa y en el bazo después de la infección con el virus de Gumboro. Se detectaron dos subpoblaciones de células B que presentaban moléculas de IgM designadas como A y B, de acuerdo al tamaño celular y a la presencia de gránulos. A pesar de que ambas subpoblaciones expresaron de manera diferente los marcadores de superficie IgM y Bu-1b, ambos grupos mostraron de manera similar los antígenos de superficie del complejo mayor de histocompatibilidad clase II. Después del desafío de aves no vacunadas con el virus de la enfermedad infecciosa de la bolsa, la subpoblación B se redujo significativamente entre siete y 21 días después del desafío, en comparación con el grupo de aves no desafiadas y con el grupo de aves vacunadas y desafiadas. Sin embargo, la reducción de la subpoblación B en la bolsa después de la exposición con el virus de Gumboro, no redujo los niveles totales séricos de IgA, IgG e IgM, tampoco afectó las células B con moléculas de IgG e IgA en el bazo. Mediante el análisis fenotípico de las subpoblaciones se identificó una expresión diferente de los antígenos de superficie Lewis<sup>x</sup>, IgM, Bu-1b y MUI78 entre las subpoblaciones. En conclusión estos son los primeros estudios que identifican dos subpoblaciones distintas de células B con moléculas de IgM en la bolsa del pollo y es el primero en describir el descenso después de la infección de la población de células B con IgM con relación a las células B con moléculas de IgA e IgG.

**Key words:** IBDV, bursa B-cell subpopulations, flow cytometry, ELISA, humoral immune response, chicken

Abbreviations: Abs = antibodies; d.a. = day of age; d.p.c. = days postchallenge; d.p.v. = days postvaccination; ELISA = enzyme-linked immunosorbent assay; FITC = fluorescein isothiocyanate; IBDV = infectious bursal disease virus; ID = infectious dose; Ig = immunoglobulin; mAb = monoclonal antibodies; MxChk = mouse anti-chicken; MHCII = major histocompatibility complex class II; NV–C = nonvaccinated–challenged; NV–NC = nonvaccinated–nonchallenged; PAB = phosphate-buffered saline containing sodium azide and bovine serum albumine buffer; PBS = phosphate-buffered saline; PE = phycoerythrin; p.c. = postchallenge; V–C = vaccinated–challenged; V–NC = vaccinated–nonchallenged

Infectious bursal disease virus (IBDV) is a bisegmented, double-stranded RNA virus belonging to the *Birnaviridae* family (7) that causes humoral and cellular immunodeficiency in chickens (29). The bursa is a major site for B-cell division, gene conversion (26), and clonal differentiation (24) and contains more than 85% IgM B-cells (9). In the bursa of Fabricius, IBDV infection results in follicular lymphoid depletion (19), a temporary reduction of proliferating immature IgM B-cells (30), and thus reduces the B-cell repertoire. Following IBDV infection, the bursa remains the functional primary lymphoid organ wherein bursal follicles are restored and B-cells are

functionally active (14), but with reduced proliferating responses (23). Recently, histologic analysis of bursas following neonatal IBDV infection revealed two distinct types of follicles in the recovering bursa, a large follicle containing a cortex and medulla and a smaller follicle without structural architecture (35). The smaller follicles appear to contain surviving, resistant B-cells, with a diversified immunoglobulin repertoire incapable of repopulating the bursa with B-cells capable of gene conversion. The larger follicle contained the Lewis<sup>x</sup> carbohydrate, a marker for the initiation of gene conversion in chicken B-cell maturation (20).

B-cell development consists of a prebursal, bursal, and postbursal phase (32). At 21 days of age (d.a.), the renewal of two of the three B-cell subpopulations in the peripheral blood is still bursa-

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Table 1. Summary of the experimental design.

Group	Vaccine <sup>A</sup>	Challenge <sup>B</sup>	Age at sampling <sup>C</sup>						
			3	10	17	24	31	38	45
I Non-vacc/Non-chall (NV-NC)	No	No	+	+	+	+	+	+	+
II Vacc/Non-chall (V-NC)	Yes	No		+	+	+	+	+	+
III Vacc/Chall (V-C)	Yes	Yes					+	+	+
IV Non-vacc/Chall (NV-C)	No	Yes					+	+	+

<sup>A</sup>No = not administered; Yes =  $10^{4.4}$  ID<sub>50</sub> S-706 IBDV commercial vaccine applied at 3 d.a.

<sup>B</sup>No = not challenged; Yes =  $10^{1.8}$  ID<sub>50</sub> Edgar IBDV challenge strain at 24 d.a.

<sup>C</sup>+ = bursa, spleen, and serum samples were taken.

dependent. In addition, the bursal B-cell subpopulations have different abilities to populate bursal compartments and peripheral tissues (22), and they differentially express surface and cytoplasmic IgM (11) and major histocompatibility complex class II (MHCII) antigens (10).

The effect of IBDV on B-cells in the bursa and spleen (6,27), and on total serum immunoglobulins (13), depends on the pathogenicity of the strains (28). Following IBDV infection at 1 d.a., total serum IgG levels are suppressed, but appear to contain increased IgM levels. A specific anti-IBDV response does not exist following infection at 1 day of age, but is moderate to high when chickens are infected at 21 d.a. (15).

The precise IBDV cell target has not been identified *in vivo*. *In vitro* studies suggest that IBDV-resistant cells may be immature (2), competent B-cells (1), or small blood lymphocytes (4). Earlier histologic evaluation of the bursa suggests the existence of bursal IBDV-resistant B-lymphocytes expressing surface determinants Bu-1b (14). In addition, there is no clear understanding of the IBDV affect on total serum immunoglobulin levels.

The objectives of this research were to examine B-cell kinetics in the bursa (primary) and spleen (secondary) lymphoid organs, in naïve and IBDV-infected chickens, using flow cytometric analysis, and to correlate these findings with the total serum immunoglobulin levels.

## MATERIALS AND METHODS

**Animals and viruses.** Female, 1 d.a., specific-pathogen-free leghorn chickens (SPAFAS, Charles River, Norwich, CT), were wing-banded and housed in separate Horsfall Bauer forced-air positive-pressure isolation units (Poultry Diagnostic and Research Center, University of Georgia) with feed and water provided *ad libitum*. Chickens were randomly allotted into four experimental groups (Table 1): (I) nonvaccinated–nonchallenged (NV-NC); (II) vaccinated–nonchallenged (V-NC); (III) vaccinated–challenged (V-C); and (IV) nonvaccinated–challenged (NV-C). At 3 d.a., 27 chickens in designated groups II and III were vaccinated ocularly with  $10^{4.4}$  infectious dose (ID<sub>50</sub>) of intermediate IBDV vaccine strain S-706 (Merial-Select Laboratories, Gainesville, GA) as recommended by the manufacturer. At 24 d.a., 18 chickens, designated groups III and IV, were orally challenged with a  $10^{1.8}$  ID<sub>50</sub> Edgar chicken embryo-adapted strain of IBDV (124-ADV-9501, National Veterinary Services Laboratories, Ames, IA).

**Antibodies.** The following primary monoclonal antibodies (mAb) were used for flow cytometry: fluorescein isothiocyanate (FITC)-labeled mouse anti-chicken (M $\alpha$ Ck) IgA (clone A-1,  $\alpha$ ), IgG (clone G-1,  $\gamma$ ), IgM (clone M-1,  $\mu$ ) (5), and R-phycoerythrin (PE)-labeled M $\alpha$ Ck Bu-1b (clone 5-11G2) against B-cell surface alloantigens (34) (Southern Biotechnology Associates, Inc., Birmingham, AL); and M $\alpha$ Ck MHCII conjugated with FITC (33) (Serotec Inc., Raleigh, NC). The antibodies (Ab) were used at dilutions recommended by the manufacturer. The anti-Lewis<sup>x</sup> Ab, MC480 (Developmental Studies Hybridoma Bank,

University of Iowa, 1.2 mg/ml), was used at a 1:500 dilution and was detected with FITC-labeled goat anti-mouse IgM,  $\mu$ -chain specific, at a dilution 1:100 (Jackson ImmunoResearch Laboratories Inc., West Grove, PA). mAb MUI78 (25) (IgG2a) was kindly provided by Dr. Richard Boyd (Monash University, Australia) and used at dilutions of 1:300, 1:30, and 1:30, respectively, and was detected with FITC conjugated anti-mouse IgG (Jackson ImmunoResearch).

**Preparation of single-cell suspensions and B-lymphocyte identification and phenotyping using flow cytometric analysis.** The bursa and spleen were aseptically collected from three chickens at 3, 10, 17, 24, 31, 38, and 45 d.a., placed in Dulbecco's modified Eagle's medium, and stored on ice. Single-cell suspensions were prepared using a 70- $\mu$ m mesh screen (Fisher Scientific, Pittsburgh, PA) as previously described (17). Cells were counted using the trypan blue exclusion method, with a hemocytometer, as previously described (8). B-cell populations were identified using unpurified, unfixed, single-cell suspensions from the bursa and spleen as previously described (3). Briefly,  $1 \times 10^6$  cells were washed twice with buffer containing  $1 \times$  phosphate-buffered saline, 0.1% sodium azide, and 0.5% bovine serum albumin (PAB buffer), incubated for 30 min on ice with 2  $\mu$ l of anti-chicken Bu-1b mAb, washed twice with PAB buffer, incubated for an additional 30 min with 1  $\mu$ l of anti-chicken IgA, IgG, or IgM, and twice-washed a second time with PAB buffer prior to flow analysis.

Bursal lymphocyte subpopulations were phenotyped using bursal cell suspensions, as described above, with additional time points of 90, 97, 104, 111, 118, and 125 d.a. The cell suspensions were stained with one of the following primary antibodies: MHCII, IgM, and Bu-1b in PAB buffer as described above (18). The antibodies anti-Lewis<sup>x</sup> and MUI78 were used as previously described (20). Briefly,  $1 \times 10^6$  cells were resuspended in 100  $\mu$ l Dulbecco's modified Eagle's medium containing 2% fetal bovine serum, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.1% sodium azide, incubated with the appropriate antibody on ice for 30 min, and washed twice. Goat anti-mouse F(ab')<sub>2</sub> IgM-FITC and IgG-FITC antibodies were utilized as secondary antibodies for anti-Lewis<sup>x</sup> and MUI78 antibodies, respectively. Samples were incubated on ice for 30 min and washed twice with medium. All wash steps included centrifugation, which was performed at  $300 \times g$  for 5 min. The cell preparations were analyzed with the FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA) and Summit<sup>TM</sup> Software (Dako Cytomation, Carpinteria, CA). A total of 20,000 events were collected per sample.

**Enzyme-linked immunosorbent assay (ELISA).** Serum was collected from three chickens per group at 3, 10, 17, 24, 31, 38, and 45 d.a. Specific IBDV antibody levels were measured with the IBDV-XR (IDEXX Laboratories, Inc., Westbrook, ME) ELISA kit. Total serum IgG, IgA, and IgM levels were measured with a commercially obtained ELISA kit (Bethyl Laboratories, Inc., Montgomery, TX). Sera from all groups were also tested against infectious bronchitis virus, Newcastle disease virus, reovirus, and chicken anemia virus using commercially available ELISA kits (IDEXX Laboratories, Inc.).

**Histopathology.** Bursa samples were taken at 31, 38, and 45 d.a. for histopathologic examination, which corresponded to 7, 14, and 21 days postchallenge (d.p.c.), respectively, in birds receiving the IBDV challenge. Briefly, bursas were fixed in 10% neutral-buffered formalin.

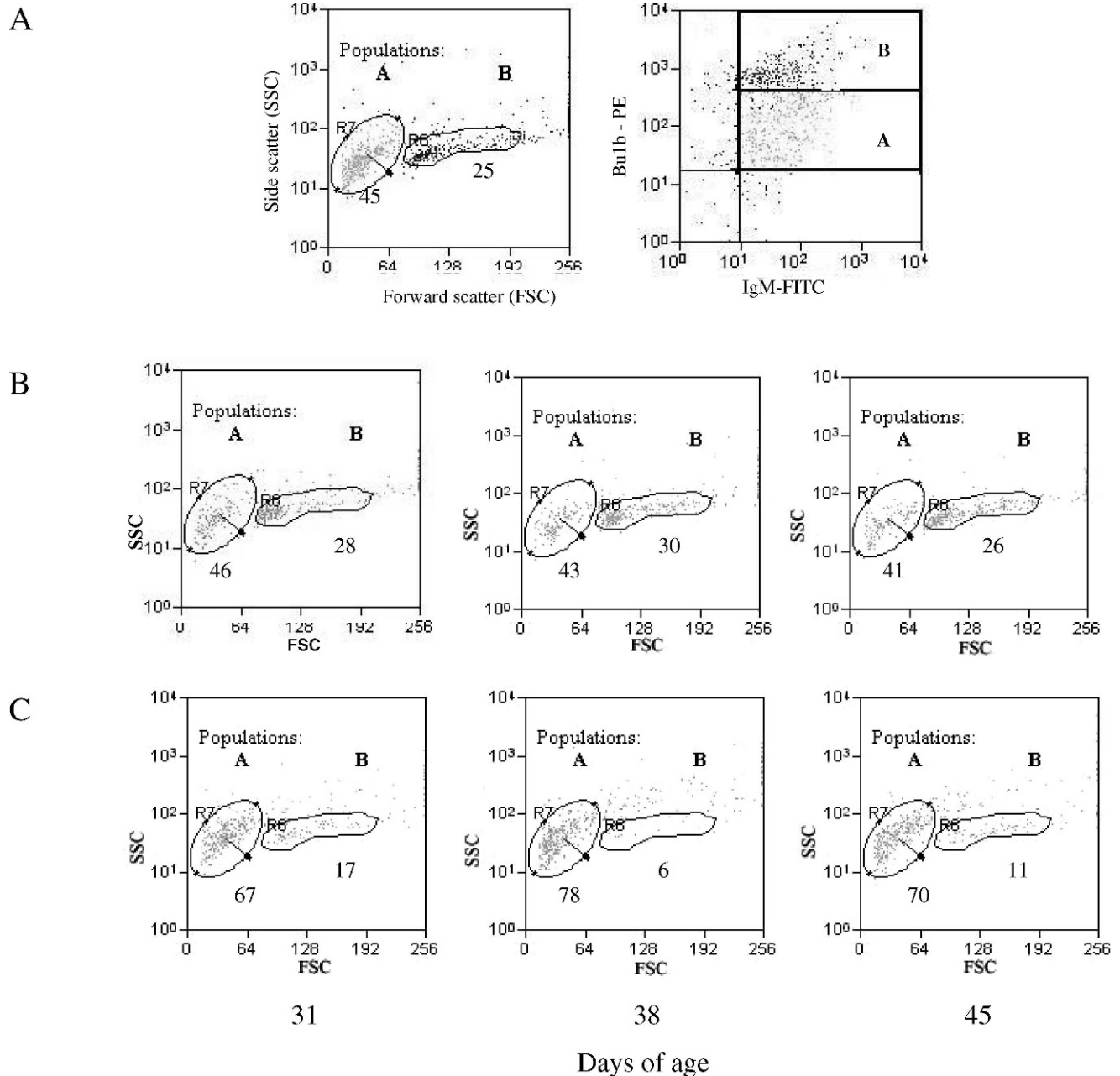


Fig. 1. Flow cytometry dot plots from different time points identifying two bursal IgM B-cells subpopulations. Bursal lymphocytes were purified at various ages and applied to flow cytometric analysis. (A) Using forward- and side-scatter analysis, subpopulations A and B were identified and further characterized by dual staining with IgM and Bu-1b antibodies, coupled to FITC and R-phycoerythrin, respectively, from NV-NC birds at 24 d.a. (B) Effect of IBDV challenge on subpopulations A and B in V-C birds at 31, 38, and 45 d.a. The vaccinated group received IBDV live vaccine at 3 d.a., and challenge was performed at 24 d.a. with the Edgar strain. (C) Decrease in subpopulation B following IBDV challenge in the NV-C groups of birds at 31, 38, and 45 d.a.

All sampled tissues were routinely processed into paraffin, and 3- $\mu$ m sections were cut and stained with hematoxylin and eosin as previously described (31).

## RESULTS

**Identification of two B-lymphocyte subpopulations in the bursa.** In the bursa, two B-cell subpopulations with different granularity and cell size, designated as A and B, respectively, were observed by applying a linear scale to the x-axis (forward scatter

[FSC]) and a log scale to the y-axis (side scatter [SSC]) to lymphocytes from NV-NC birds. These subpopulations segregated following staining with IgM and Bu-1b antibodies (representative histogram shown at 24 d.a.; Fig. 1A). The A subpopulation was identified as being smaller than subpopulation B, but as having equivalent granularity. Subpopulation B appeared to express an increased amount of Bu-1b antigen as compared to subpopulation A, based on forward-scatter analysis. However, both A and B expressed relatively equal amounts of IgM (see Fig. 1A, IgM-FITC).

**Effect of IBDV challenge on B-lymphocytes in bursa of subpopulation A and B.** Using forward- and side-scatter analysis of

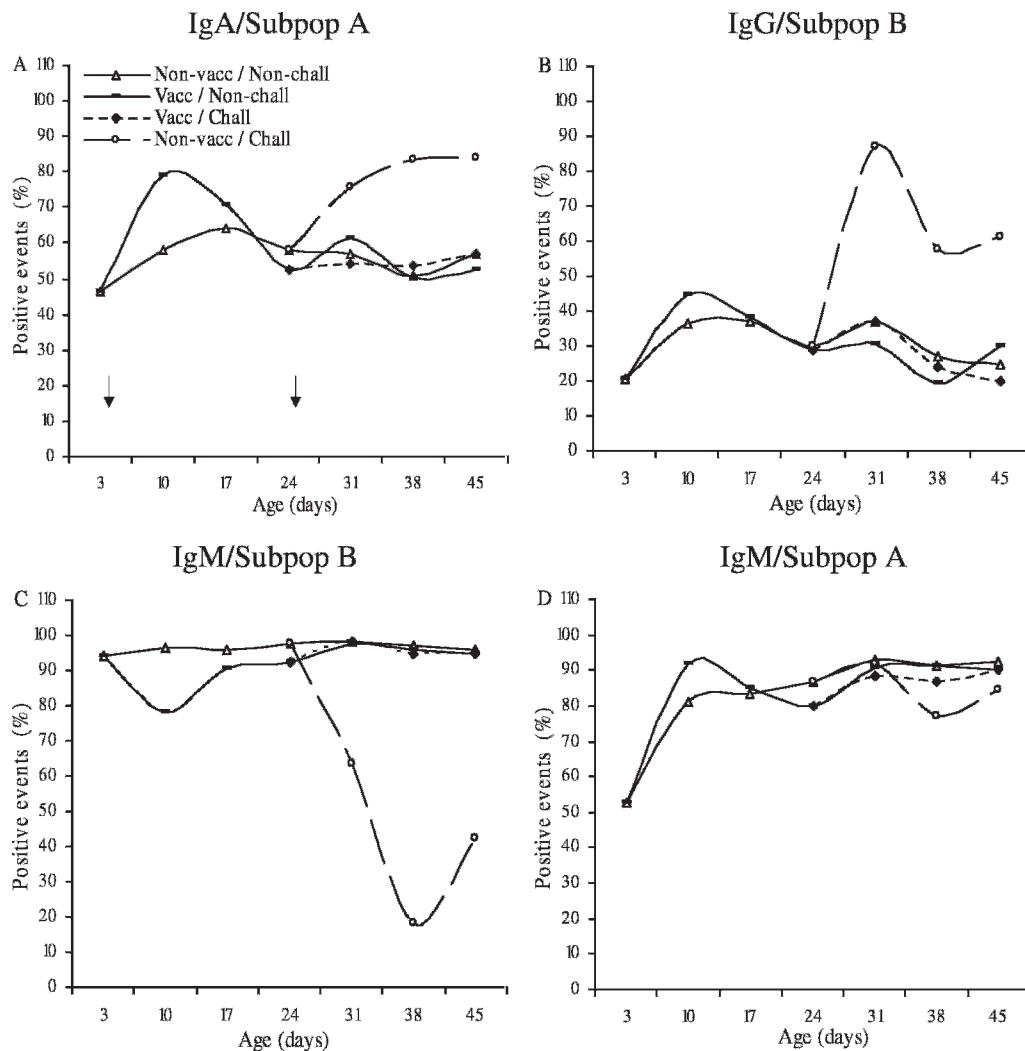


Fig. 2. Kinetics of IgA, IgG, and IgM-bearing B-lymphocytes in bursa measured with flow cytometry between 3 and 45 d.a. in all groups. (A) IgA B-cells from subpopulation A, (B) IgG B-cells from subpopulation B, (C) IgM B-cells from subpopulation B, and (D) IgM B-cells from subpopulation A. Arrows indicate IBDV vaccination (3 d.a.) and challenge (24 d.a.).

bursal lymphocytes, the effect of IBDV challenge on subpopulation A and B was determined. Following challenge with the Edgar strain, a similar proportion of subpopulation A and B was observed in the V-C group of birds at days 31, 38, and 45 d.a. (Fig. 1B) when compared to the NV-NC birds (Fig. 1A). However, when unvaccinated birds were challenged, a noted decrease in the proportion of subpopulation B was evident (Fig. 1C). The percent of stained lymphocytes in subpopulation B dropped from 17% at day 7 postchallenge (p.c.) to 6% at day 14 p.c., but increased to 11% at day 21 p.c. In the groups of IBDV-unchallenged birds, the NV-NC and V-NC groups, the proportion of subpopulations A and B was similar to the V-C birds on the same days tested (data not shown).

**Effect of IBDV vaccination and challenge on bursal IgA, IgG, and IgM B-lymphocytes.** In the bursa, staining of subpopulation A IgA cells increased in the V-NC group at 7 days postvaccination (d.p.v.) and in the NV-C group between 7–21 d.p.c. (Fig. 2A), corresponding with the introduction of either vaccine (V-NC at 3 d.a.) or challenge (NV-C at 24 d.a.) virus, respectively. No differences were observed in subpopulation B IgA cells, regardless of the presence or absence of IBDV (data not shown). However, subpopulation B IgG cells increased in the NV-C group between 7–

21 d.p.c. (Fig. 2B), but remained constant between the other groups. No differences were observed in subpopulation A IgG cells between different groups (data not shown). However, in the NV-C group, IgM-positive cell staining decreased at 7 d.p.c., appeared near depletion at 14 d.p.c., and began to increase at 21 d.p.c. (Fig. 2C). Interestingly, a similar, yet less-pronounced effect was observed in the V-NC group following vaccination, wherein the subpopulation B IgM cells were initially decreased at 7 d.p.v. with IBDV, but returned to the levels observed in nonvaccinated groups. These observations correlated with results observed and are shown in Figure 1C. Interestingly, staining of subpopulation A IgM cells did not dramatically change over time between the four study groups tested, regardless of IBDV infection (Fig. 2D).

**Effect of IBDV vaccination and challenge on B-lymphocytes in spleen.** The B-cells from the spleen were presented as a single population when dually stained with Bu-1b and IgM mAb, and were only observed within population A (Fig. 3A). Approximately 75% of cells in population A stained positive for Bu-1b, whereas only 39% stained positive for IgM. In general, no differences were observed for IgA staining between any of the groups tested (data not shown). No major differences were observed in IgG B-cells in the spleen between the NV-NC, V-C, and V-NC groups, with percent

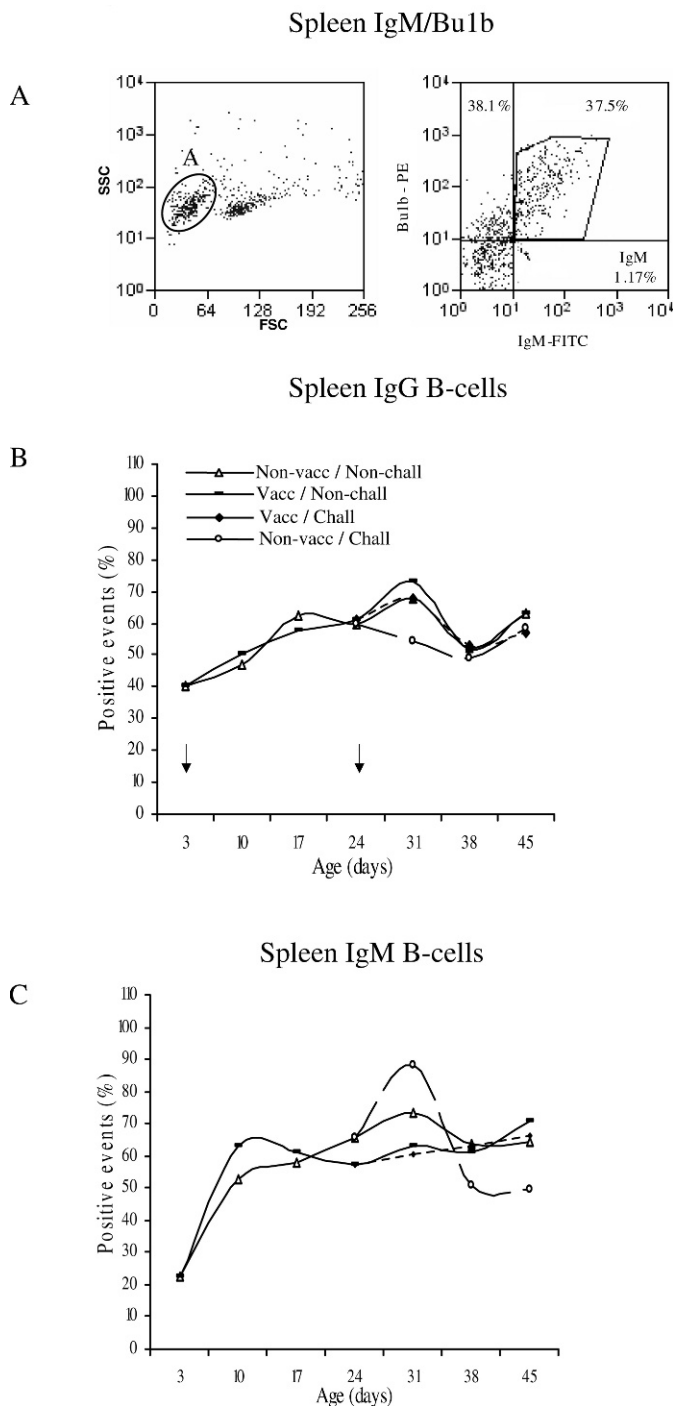


Fig. 3. Kinetics of IgG and IgM-bearing B lymphocytes in spleen, measured with flow cytometry between 3 and 45 d.a. in all groups. (A) Using forward- and side-scatter analysis, B-lymphocytes were identified in the spleen and further characterized by dual staining with IgM and Bu-1b antibodies, coupled to FITC and R-phycoerythrin, respectively, from NV-NC birds at 24 d.a. (B) Percent positive IgG B-cells in all groups. (C) Percent positive IgM B-cells in all groups.

staining increasing from approximately 40% at 3 d.a. up to approximately 65% at 31 d.a. Following Edgar challenge, a noticeable decrease was observed in the NV-C group in IgG B-cell staining at 7 d.p.c. (Fig. 3B). Interestingly, this group displayed an increase in IgM B-cells, which increased at 7 d.p.c. (e.g. 31 d.a.)

to nearly 90%, and then decreased at 14 d.p.c. and 21 d.p.c., as compared to the other groups (Fig. 3C).

**ELISA.** Total serum IgA was elevated in the V-C group between 7–34 d.p.v. and in the NV-C group between 7–21 d.p.c. (Fig. 4A). Total serum IgG was decreased in the NV-C group at 7 d.p.c. and elevated between 14–21 d.p.c. (Fig. 4B). Total serum IgM was elevated only in the V-NC group at 7 d.p.v. and in the NV-C group at 7 d.p.c. (Fig. 4C). Specific IBDV seroconversion in the V-NC group was observed at 14 d.p.v., peaked at 28 d.p.v., and was undetectable at 34 d.p.v. The IBDV titer in the V-C group peaked at 7 d.p.c., was higher than in the V-NC and NV-C groups, and was negative at 21 d.p.c. The IBDV titer in the NV-C group peaked at 7 d.p.c. and was higher than in the V-NC group through 21 d.p.c. (Fig. 4D). Interestingly, at 7 d.p.c., the NV-C group displayed the highest specific IBDV titer while the total serum IgG was at its lowest level.

**Histopathology.** As expected, severe bursal damage was observed following challenge with the Edgar strain of IBDV in the NV-C group. Remnants of bursal follicles with epithelial cells, fibroplasias, and macrophage infiltration, along with diffuse lymphoid depletion, were visible with severe lymphocytolysis in the bursas at 7 and 14 d.p.c. (Fig. 5). Follicular regeneration was observed in this group at 21 d.p.c. with both large and small follicles evident. No pathologic changes following challenge were observed in the NV-NC, V-NC, and V-C groups during the same time period (Fig. 5).

**Phenotypic characteristics of subpopulation A and B bursal B-lymphocytes.** Bursal subpopulation A and B were phenotyped using B-lymphocyte surface-expressed antigens and flow cytometric analysis between 3 and 125 d.a.

The MUI78 percent positive cells at 3 d.a. from subpopulation A and B was 32% and 40%, and at 125 d.a. was 35% and 78%, respectively (Fig. 6A). While subpopulation A tended to decrease expression over time, subpopulation B tended to increase expression over time. In the two subpopulations, the percent MHCII-positive cells was close to 95%, and no differences were observed between both subpopulations between 3–125 d.a. (Fig. 6A).

No differences in the percent Lewis<sup>x</sup>-positive cells were observed between subpopulation A and B during 3–31 d.a. (Fig. 6B). However, from 38 to 125 d.a., the percent positive cells in both subpopulations displayed decreased staining. In subpopulation B, staining was no less than 76%; however, in subpopulation A, only 40% of the cells were positive at the last time point at 125 d.a. (Fig. 6B).

## DISCUSSION

In the bursa, two B-cell subpopulations, designated as A and B with different flow cytometry profiles and kinetics, were identified. A marked reduction in subpopulation B bursal IgM cells following IBDV challenge was observed. The results observed here indicate a susceptibility of this cell population to IBDV infection, either through direct infection or secondary factors. It is not clear whether this cell type is infected with virus, or is responding to apoptotic effects of surrounding IBDV-infected B-cells. It was previously suggested that the age of IBDV infection is a critical factor for the humoral immune response (6,14) and disease outcome (15).

Subpopulations A and B IgM cells in the V-C group, following challenge, were not affected by IBDV challenge, most likely due to the protective effects of the neutralizing antibodies produced following vaccination. Although not addressed in these studies, the contribution of T-cell immunity following live-virus vaccination

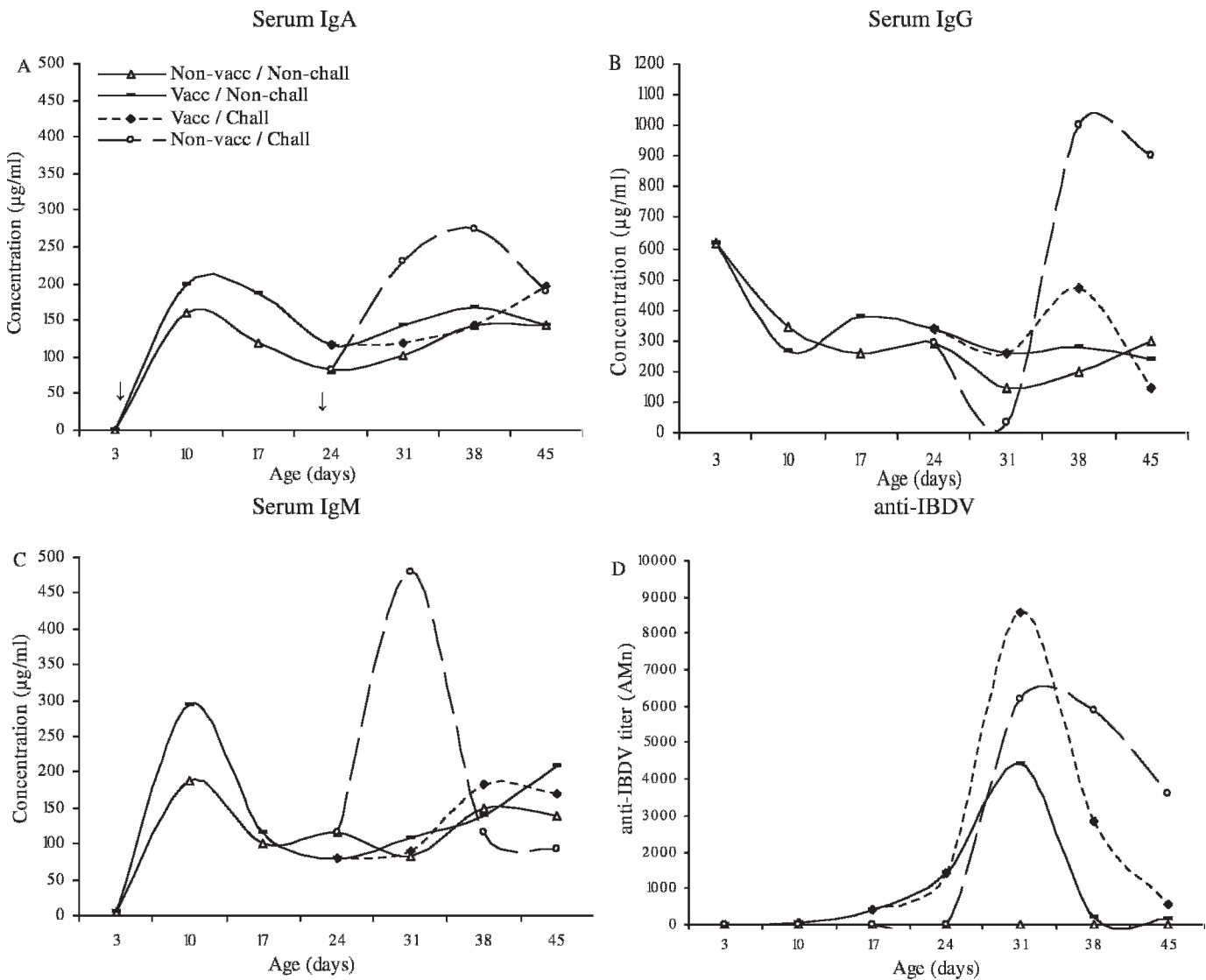


Fig. 4. Total serum immunoglobulin levels in all groups of birds. (A) IgA antibody concentrations, (B) IgG concentrations, (C) IgM concentrations, and (D) anti-IBDV titers. Arrows indicate IBDV vaccination and challenge.

must also be considered as reducing the overall bursal damage we observed in the NV-C group.

The differences observed in B-cell repopulation between the NV-C and V-C groups may also be attributed to lower numbers of susceptible cells in the bursa at 3 d.a. as compared to 24 d.a. and to differences in virulence of the strains used for vaccination and challenge. Bursal follicle restoration and increased IgM cells in subpopulation B at 21 d.p.c. were evident in the NV-C birds, indicating recovery from disease. However, the ability of these B-cells to respond to antigen is unknown.

Bursal follicle B-cell repopulation following challenge of nonvaccinated birds may be indicative of IBDV-resistant immature B-cells, which were at an earlier developmental stage in subpopulation B. The main support for this concept is the increased Lewis<sup>x</sup> expression in subpopulation B as compared to A, implying gene conversion capabilities, although both subpopulations contained Lewis<sup>x</sup>-negative B-cells. The Lewis<sup>x</sup> initial expression coincides with the initial immunoglobulin gene conversion and is subsequently down-regulated with the bursal lymphocytes maturation (18,21).

The major histocompatibility complex has been linked to immune response against IBDV, and MHCII was suggested to

restrict a T-cell dependent secretory antibody response (16). The majority of the cells in both subpopulation A and B were MHCII positive between 3–125 d.a. MHCII is expressed on antigen-presenting cells and is involved in presentation and recognition by T-cells (33). MHCII expression coincides with IgM expression and increases during the bursal embryonic phase of B-cell differentiation (10).

Following Edgar challenge at 24 d.a. in the NV-C group, increased levels of subpopulation A IgA B-cells and subpopulation B IgG B-cells, respectively, were observed in the bursa. Whether the overall number of these B-cells increased, or simply appeared elevated due the decrease in IgM B cells, remains to be determined. However, in a previous study using flow cytometry, no significant difference in IgA cells was observed between V-C birds and NV-NC birds, implying that the increases observed in these studies may be due to the loss of IgM B-cells in the sample (27). However, differences in the results between that study and these may be due to the strain differences and the infectious dose used in the experiments.

In the spleen, the percent positive B-cells did not change between the V-NC and NV-NC groups. However, a slight decrease in IgM

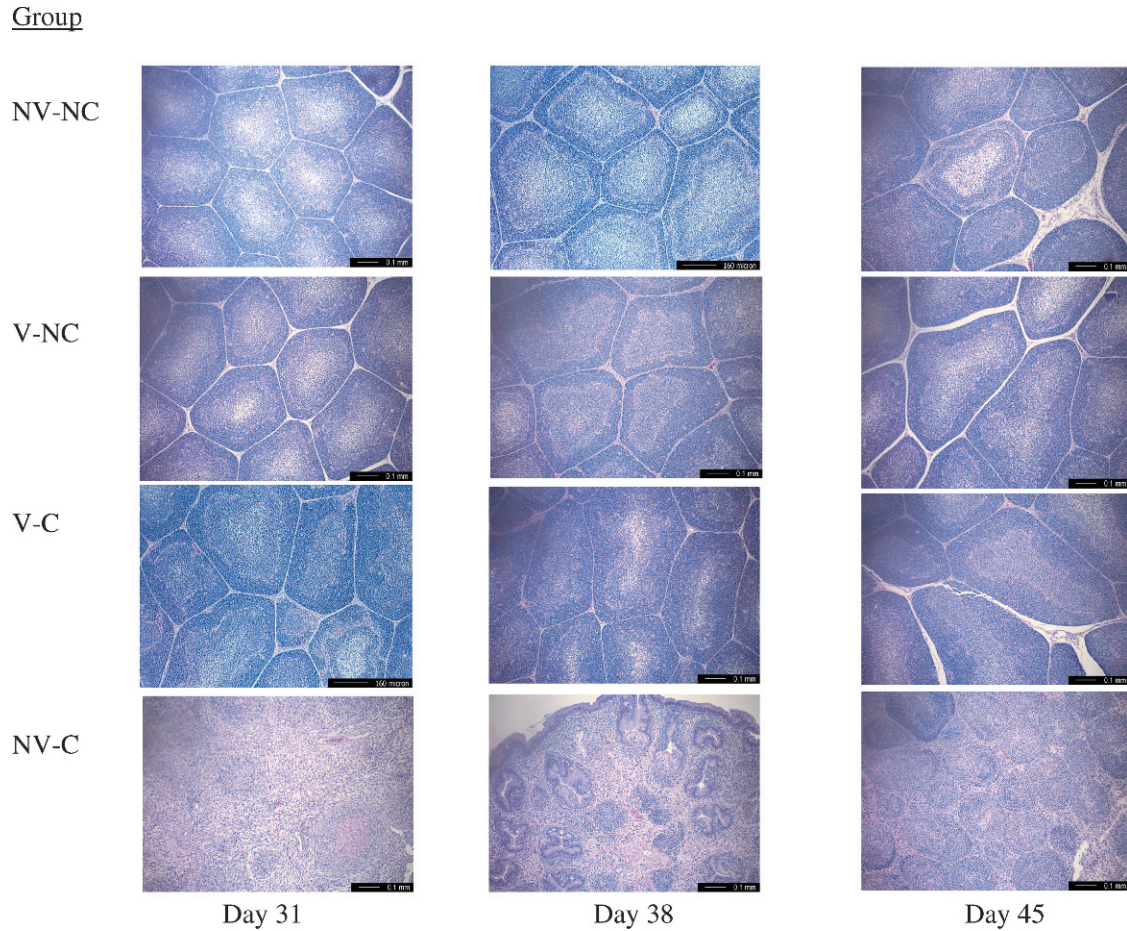


Fig. 5. Histologic evaluation of bursa from NV-NC, V-NC, V-C, and NV-C groups of birds. All samples were taken from birds at 7, 14, and 21 d.p.c. and stained with hematoxylin and eosin. The vaccinated (V) groups were vaccinated at 3 d.a. and the challenged (C) groups received the Edgar strain at 24 d.a. Remnants of bursal follicles with epithelial cells, fibroplasias, and macrophage infiltration, along with diffuse lymphoid depletion, were visible with severe lymphocytolysis in the bursas from the NV-C group at 31 and 38 d.a. Follicular regeneration was observed in this group at 45 d.a.

B-cells was observed in the NV-C group at 7 d.p.c., which correlated with a previous report (6). It has also been reported that both IgG and IgM B-cells in the spleen are decreased post-IBDV infection, although we did not observe this decrease in IgG cells (12). The varied observations between the studies may be due to the differences in antibody reactivity between the previous experiments.

Major differences in IgA, M, and G immunoglobulin levels were observed between the V-NC and NV-C groups. Taken together, these results suggest a failure of IgM B-cells to undergo gene conversion following challenge, resulting in an accumulation of IgM and a decreased conversion to IgG antibodies. The specific IBDV seroconversion in the NV-C group was elevated faster and higher,

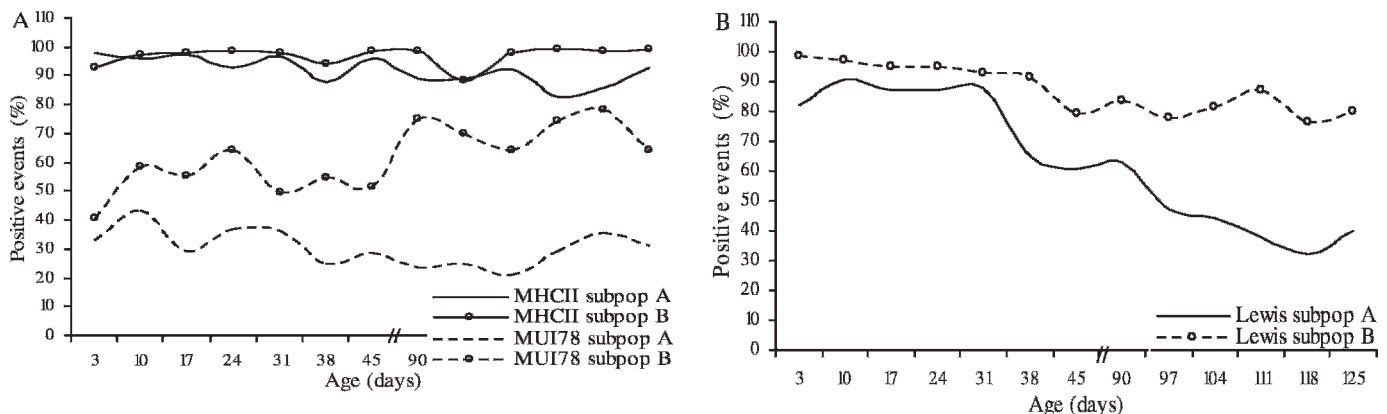


Fig. 6. B-lymphocyte phenotype expression of subpopulation A and B, between 3 and 125 d.a., from bursa of NV-NC chickens using surface marker antigens and flow cytometry. (A) Comparison of MHCII and MUI78 surface staining and (B) Lewis<sup>x</sup> surface staining.

and persisted longer, than in the V-NC group. In the V-C group, IBDV titers decreased quickly following challenge, when compared to the other two groups, likely due to the clearance of the challenge virus from the vaccinated host.

In conclusion, this is the first report to identify and phenotype two distinct B-cell subpopulations in the bursa, following IBDV infection, by using flow cytometry analysis. Subpopulation A remained at constant levels within each group, regardless of vaccine-challenge status and, thus, appeared resistant to IBDV challenge. In contrast, subpopulation B declined in NV-C, but returned to normal levels over time, suggesting this subpopulation is susceptible to IBDV infection. The reduction of subpopulation B in the bursa did not reduce the total serum immunoglobulin levels, nor did it affect IgG and IgA B-cells in the spleen. Future experiments will be designed to identify whether or not the B-cell subpopulations become infected with IBDV. While further work is needed to characterize these two subpopulations, results presented here provide a platform to better understand the dynamics of IBDV infections on B-cell development and immunity in poultry.

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#### ACKNOWLEDGMENTS

We would like to thank Mr. Fred Smith and the animal care staff at the Poultry Diagnostic and Research Center, University of Georgia. This research was supported by the Veterinary Medical Agriculture Research Fund at the University of Georgia.